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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Shah et al.

Serial No.: 09/891,983

Filed: June 26, 2001

For: METHODS FOR THE SIMULTANEOUS DETECTION OF HCV ANTIGENS AND HCV ANTIBODIES

Case No.: 6821.US.01

Examiner: Wortman, D.

Group Art Unit: 1648

Certificate of Mailing under 37 CFR §1.8(a): I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the:

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Date of Deposit: 1-15-04  
*Kimberly A. Iorio*  
Kimberly A. Iorio

DECLARATION UNDER 37 C.F.R. § 1.131

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

We, DINESH O. SHAH, GEORGE J. DAWSON, A. SCOTT MUERHOFF, LILY JIANG, ROBIN A. GUTIERREZ, THOMAS P. LEARY, SURESH DESAI AND JAMES L. STEWART, citizens of the United States of America and residents of either Illinois or Wisconsin, do declare and say that:

We are co-inventors of the above-referenced application for patent filed on June 26, 2001.

In the Office Action of April 29, 2003, claims 13 and 14 are rejected under 35 U.S.C. 102(e) as being anticipated by Chien et al. (U.S. Patent Publication No. 2002/0192639

A1). Additionally, claims 13 and 14 are rejected under 35 U.S.C. 102(e) as being anticipated by Bahl et al. (U.S. Patent Publication No. 2003/0049608 A1).. Further, claims 13 and 14 are rejected under 35 U.S.C. 102(e) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Aoyagi et al. (U.S. Patent Publication No. 2002/0173493 A1). Additionally, claims 8-11 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Aoyagi et al. (U.S. Patent Publication No. 2002/0173493 A1). Further, claims 8-11 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chien et al. (U.S. Patent Publication No. 2002/0192639 A1). Also, claims 8-12, 14 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bahl et al. (U.S. Patent Publication No. 2003/0049608 A1) in view of Chien et al. (U.S. Patent Publication No. 2002/0192639 A1).

We, conceived and reduced to practice, in the United States, the invention claimed in claims 13 and 14 prior to the priority date (i.e., the date of filing of the provisional application) of Chien et al. (i.e., June 15, 2000), prior to the priority date (i.e., the date of filing of the provisional application) of Bahl et al. (i.e., March 28, 2001) and prior to the filing date of Aoyagi et al. (i.e., April 26, 2002). Further, we conceived and reduced

to practice, in the United States, the invention claimed in claims 8-11 and 15 prior to the filing date of Aoyagi et al. (i.e., April 26, 2002) and prior to the priority date of Chien et al. (i.e., June 15, 2000). Additionally, we conceived and reduced to practice, in the United States, the invention claimed in claims 8-12, 14 and 15 prior to the priority date of Bahl et al. (i.e., March 28, 2001) as well as Chien et al. (i.e., June 15, 2000). These assertions are evidenced by the following:

Attached Exhibit A illustrates that, prior to June 15, 2000 (i.e., the priority date of Chien et al. and the earliest date of the documents cited above), we developed a method for the simultaneous detection of HCV antigens and HCV antibodies in a test sample. In particular, as evidenced by Exhibit A, in one embodiment, the HCV antigens were to be captured on a solid phase, and then the captured antigens were to be detected with an antibody (e.g., monoclonal antibody) labeled with a reporter molecule. Further, the solid phase was to be coated with various HCV proteins (e.g., NS3, NS4 and fragments of the core protein) in order to capture HCV antibodies. The antibodies would then be recognized by a second antibody (e.g., goat anti-human IgG) labeled with a reporter molecule.

Further, Exhibit A also illustrates a schematic view of the assay. In particular, the figure establishes how the antibodies in the test sample are to be detected as well as how the core antigens are to be detected using conjugated monoclonal antibodies.

Exhibit B illustrates that prior to the June 15, 2000 priority date of Chien et al., we carried out the assay and obtained positive data. In particular, Exhibit B illustrates various reagents used in the assay (i.e., those coated on the solid phase) and evidences that upon running the assay, results were obtained indicating that one could detect HCV antigen and HCV antibody simultaneously in a sample.

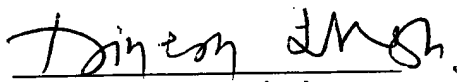
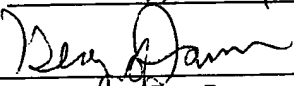
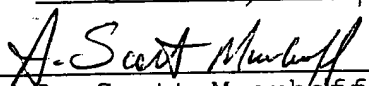
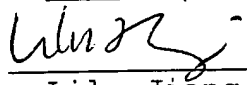
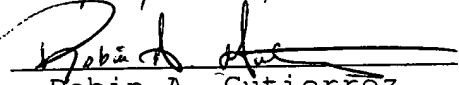
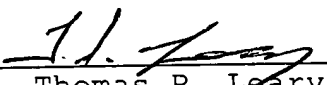
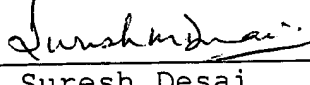

In summary, the attached Exhibits establish that the claimed invention was conceived of and reduced to practice, prior to the priority date of Chien et al. (i.e., June 15, 2000) as well as the subsequent dates of Bahl et al. and Aoyagi et al.

Although all the dates on Exhibits A and B have been blocked out, such dates are prior to June 15, 2000.

We declare further that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that

willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the instant application or any patent issuing thereon.

Respectfully submitted,

- 1)   
Dinesh O. Shah  
Date: Jan 7, 2004
- 2)   
George J. Dawson  
Date: Jan 7, 2004
- 3)   
A. Scott Muerhoff  
Date: Jan 7, 2004
- 4)   
Lily Jiang  
Date: 1/7/2004
- 5)   
Robin A. Gutierrez  
Date: 1/9/2004
- 6)   
Thomas P. Leary  
Date: 01/07/04
- 7)   
Suresh Desai  
Date: Jan 7, 2004
- 8)   
James L. Stewart  
Date: 1/8/04

PROJECT HCV antigen test

EXP. OR CODE NO. \_\_\_\_\_

There have been recent indications that HCV core proteins can be detected in serum of HCV infected individuals, most notably the publications from Toron Corporation (Tanaka et al, Journal of Hepatology 1995 23: 742-745 and Aoyagi et al, in the Journal of Clinical Microbiology 1999 37:1802-1808. There have been no published disclosures pertaining to an antigen/antibody combo test for detection of exposure to HCV to date. There are several possible methods for devising a combo HCV test, allowing detection of both antibodies and antigens associated with exposure to HCV. Current antigenic targets for the antibody test include recognition of viral proteins derived from several different open reading frames of the virus including core + envelope proteins as well as proteins from nonstructural regions designated as NS (nonstructural) 2, NS<sub>3</sub>, NS<sub>4</sub> and NS<sub>5</sub>. Commercialized tests currently utilize HCV proteins from NS<sub>3</sub>, NS<sub>4</sub> and/or NS<sub>5</sub>.

(Continued on page 5)

SIGNATURE Greg Jones

DATE \_\_\_\_\_

WITNESSED BY Willy King

DATE \_\_\_\_\_

PROJECT \_\_\_\_\_

HCV antigen test

EXP. OR CODE NO. \_\_\_\_\_

A potential combo test would continue to utilize one or more vitally derived proteins from HCV for antibody detection but would also employ antisera generated against HCV proteins to develop an antigen sandwich assay which captures HCV proteins on a solid phase (nitrocellulose, microparticles, polystyrene plate or beads) and then further detects the captured protein with a labeled antibody. One of the most likely targets for detection of HCV antigens is the HCV core protein. While Boreen corporation has clearly demonstrated utility of an HCV antigen test, there has been no clear indication of a combo test being developed.

For this reason, the following proposal is made - Abbott Labs would develop an antibody / antigen combo test, allowing simultaneous detection of antibodies & antigens associated with exposure to HCV. In one example of this combo assay, a solid phase would be coated with HCV protein (NS3, NS4 and fragments of the HCV core protein) and also coated with antibodies to HCV. (continued on page 6)

SIGNATURE \_\_\_\_\_

Jose Jaim

DATE \_\_\_\_\_

WITNESSED BY \_\_\_\_\_

Willy King

DATE \_\_\_\_\_



PROJECT HCV Antigen test

EXP. OR CODE NO. \_\_\_\_\_

This solid phase would capture antibodies to HCV or would also capture HCV proteins (e.g. core protein). The captured antibodies (that were captured due to antibodies binding to HCV proteins) would be recognized by a second antibody (e.g. goat anti-human IgG) that is labeled with a reporter molecule (horseradish peroxidase, acidinium, biotin, etc.) allowing detection of antibodies directed against the solid phase-bound proteins derived from HCV.

The captured antigens would be recognized in one example of the combo assay by specific antibodies (e.g. monoclonal antibodies) against the core protein. This specific antibody would be labeled with a reporter molecule (horseradish peroxidase, biotin, acidinium) to allow detection of the bound antigen.

One of the important differences between the combo test for HCV and HIV as proposed here is that one continues to be able to detect antibodies to core and core antigens at the same time. In order to do this successfully, the core protein needs to be re-engineered. See page 7

SIGNATURE [Signature] DATE \_\_\_\_\_WITNESSED BY [Signature] DATE \_\_\_\_\_

PROJECT HCV antigen test

EXP. OR CODE NO. \_\_\_\_\_

The core protein of HCV consists of 91 amino acids. For detection of antibodies to HCV only segments of the core molecule would be needed. For example it is known that there are epitopes associated with antibody detection at amino acids 9-88 based on 15kDa protein. Thus, in one version of a combi assay the solid phase would be coated with NS3, NS4 & NS5 proteins and a modified core protein (containing needed epitopes) as well as one or more monoclonal antibodies (or possibly polyclonal antibodies to core). See Figure on page 7. Further the conjugates would recognize the bound antibodies (captured with specific antigen) or bound antigens (captured with specific antibodies). The candidate core proteins would be: recombinant core proteins (aa 1-100, aa 1-20, aa 8-89, aa 9-99 etc) with monoclonal antibodies recognizing epitopes outside of the regions recognized by antibodies in human serum. Alternatively one could use peptides 6mers or greater covering major epitopes between amino acids 1-100. Further, the antigens on the solid phase could be re-engineered to include amino acid substitutions, deletions, etc.

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DATE \_\_\_\_\_

WITNESSED BY [Signature]

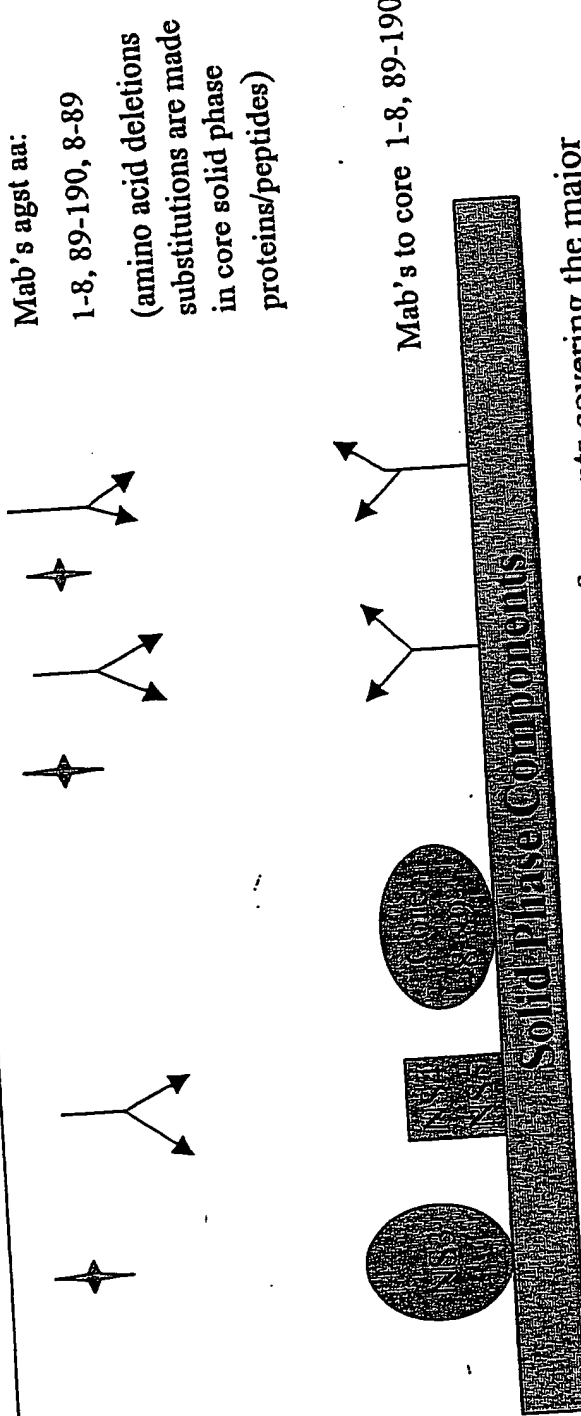
DATE \_\_\_\_\_

**Schematic of Potential HCV Combo Antibody/Antigen Test**

**Liquid Phase Conjugates:**

Goat anti-human conjugate  
- detect antibodies to core, NS3-5

Conjugated mab's to core  
- detects core antigen in serum  
- conjugates must not recognize solid phase core



Mab's agst aa:  
1-8, 89-190, 8-89  
(amino acid deletions  
substitutions are made  
in core solid phase  
proteins/peptides)

Mab's to core 1-8, 89-190

\*Recombinant core aa 8-89 may appear as a single entity or as fragments covering the major epitopes...sequences may be modified by deletions, substitutions between aa 8-89 that do not perturb major epitopes. Synthetic peptides covering aa 8-89 would also be a viable alternative.


SIGNATURE \_\_\_\_\_

DATE \_\_\_\_\_

WITNESSED BY \_\_\_\_\_

DATE \_\_\_\_\_

*Jensen*

PROJECT

*HCV combo Assay*  
*Ab, Ag*

EXP. OR CODE NO.

*Blended up and conj.*

*This is the first demonstration of a combination antibody/Ag test for HCV.*

*cont. on pge #10*

L JUN 10

21:29:19

N/A

A

1073

V COMBO ASSAY

**Blended Up and Blended conjugate**  
Up: HC31 (DF=3 Coating conc: 200ug/ml) + C11-14 (0.09% 0.4um)  
Conjugate: C11-10 (100ng/ml 1:16) + 6A52B (1/5 dilution in HIV combo CD)  
Washes: HIV ag transfer wash Dev lot 5/ final wash : HCV Ag prep.  
SDB: 6A52Q  
Up diluent: 18488 HCV Ab assay up diluent  
S/A configuration: HCV

Samples	SubA	SubB	Combo Assay Mean counts	P/N	Ab Assay Mean Counts 08/28/00	P/N	Ag Assay Mean counts 08/24/00	P/N
PC (Ab)	1502	1923	1712.5	2.17	33952	8.64	79	55.82
NC (Ab)	808	852	790		3930.17			86.36
	755	745	719	0.91	4816.75	1.23	681.5	1.41
98800	718	1083	1110	11.91	36800.87	37.48	2845	1.41
Panel A	1167	9035	9410	10.26	147307.5		4708.5	1.41
E2 1/20 dli	9785	8237	8104.5	3.82			5071	1.41
Promed 8992181	7872	3639	3084.5	8.86			4258	1.41
PC JV 018929	2550	5290	6258.5	1.10			2853	1.41
PO JV017220	5227	899	870.5	2.36			4829	1.41
Sure-Tec panel #3	842	1773	1863.5		1427	0.36		
4	1954	2463	2507.5		2058.5	0.52		
6	2552	3507	3558.5		1716.5	0.43		
6	3608	3120	3001		1607.5	0.36		
7	2882	2280	2172.5		1671.5	0.43		
8	2055	3707	3707		1885	0.42		
8								

1277

21611

*As the first Blend up and conj. results are encouraging. Dilute conj more for next run.*

SIGNATURE *[Signature]*

DATE

WITNESSED BY *[Signature]*

DATE

PROJECT HCV Combo Assay

EXP. OR CODE NO. Cont. from page # 8

DESCRIPTION OF PANEL MEMBERS -

NC - negative control - pooled plasma individually screened as negative for HCV antibodies by a commercialized assay- Code: 6A52E. Prism HCV Ab Assay Negative Calibrator.  
PC - positive control - pooled anti-HCV positive plasma diluted in negative control . Code: 6A52F. Prism HCV Ab Assay Positive Calibrator.

99800 - Plasma (human) Recalcified Negative Bulk.

Panel A - an anti-HCV positive plasma that has been diluted in negative control to provide a mid range sample to cutoff in the PRISM antibody assay.

E2 1/20 - an anti-HCV positive sample that has been diluted in negative control - the E2 antibody panel was utilized to titrate the potency of HCV E2 antigen coated microparticles

Promed 9992161 - an antibody positive sample obtained from ProMedX (Plainville, MA)

PC JV 16929 - Sero-Tec HCV RNA positive human plasma .  
PC P JV17220 - Sero-Tec HCV RNA positive human plasma .

SeraTec Panel members 3-9 - serial bleeds obtained from a plasma donor identified at SeraTec as being anti-HCV negative and HCV antigen positive.

A panel of specimens previously characterized as having antibodies to HCV or being negative for antibodies to HCV but positive for HCV RNA and HCV antigens were tested in a preliminary HCV combination antibody/antigen test.

Reagents utilized in combo test

Microparticles specific for HCV antigen detection (up's coated with C11-14 as described on RB: 67093 page 100 ) and microparticles specific for HCV antibody detection (up's coated with HCV recombinant protein HC 31 as described on RB: 68160page 2 ) were blended to produce a solid phase that would allow simultaneous detection of HCV antibodies and HCV antigens in a single reaction well. (The blended microparticles contained 0.19% solids, representing a mixture of 0.09% up's coated with C11-14 and 0.1% coated with HC31). The conjugates were also a mixture of two separate acridinium labeled proteins. Acridinium labeled C11-10 was utilized for HCV antigen detection (recognizing HCV antigens captured on the C11-14 microparticles) and an acridinium labeled monoclonal antibodies against biotin -labeled goat anti-human IgG (presented as a pre-complex - see RB: 52226m301 ) was utilized to detect human anti-HCV IgG bound to the HC-31 coated microparticles.

Results

The panel described above was run on 3 different PRISM-based assays. One of the assays detected HCV antibodies, a second test detected HCV antigens and a third test (the combo assay) detected both HCV antibodies and HCV antigens.

Sampels have a positive to negative ratio (P/N ) ratio of 3.0 or greater were considered positive. The data presented in the table on RB68160page 8 indicate that the combo assay allows detection both of antibody positive samples (e.g. panel E2 1/20, ProMed 9992161, PC JV 016929 and PC JV 17220) and HCV antigen positive samples (Sera Tec panel members 5-9). Thus, this single combo assay performed in a single reaction well detects most of the samples that were positive in two separately performed assays, the HCV antibody test and the HCV antigen test. This is the first demonstration of a combo HCV antibody / HCV antigen test at Abbott Laboratories, and is the first example of the HCV antibody /antigen combo test ideas presented in Redbook 61,959: pages 1-8. Other iterations of the HCV combo test will be presented over the next several weeks/months.

S. E. EPT.

Willy P. J. P.  
more description etc

SIGNATURE Willy P. J. P. DATE \_\_\_\_\_

WITNESSED BY Cathy Koon DATE \_\_\_\_\_

RESEARCH DEPARTMENT

PROJECT HCV combo Assay \*

EXP. OR CODE NO. \_\_\_\_\_

Title: HCV combo Assay: Blended up and Blended conjugate

Purpose: To blend the HCV core peptide coated ups, NS3NS4 coated up, c11-14 coated ups together and c11-10, aHigG Acr\* conjugate together for HCV combo first demonstration.

Materials and Samples  
RB: 68160001 and 68160011.

(Core peptide Ag + NS3NS4 for Ab Detection)  
c11-14+Ab coated up for Ag Detection)

Preparation:  
Add Avidin 11-28 (df=20) and NS3NS4 (df=10) and c11-14 (0.09% seradyn)  
Add conjugate c11-10 (50ng/ml) and aHigG Acr\* (10ng/ml)

Results:

HCV Combo (11-28, NS3NS4, c11-14, c11-10, aHigG) 9 12

Conclusions:  
The combo assay successfully detected all the Ab pos. samples and Ag positive samples.

Next Steps:  
Dilute the aHigG conjugate to 7ng/ml and 2 ng/ml

1023 6 A N/A 09/12/00 14:20:02 L JIANG  
HCV COMBO 11-28, NS3NS4  
c11-14 c11-10 aHIGG

HCV Combo Assay  
Blended ups: HCV Core Bio-11-28 (DF=20)+ NS3NS4 HCV Ag (DF=10)+ C11-14 (0.09%)  
Conjugate: c11-10(50ng/ml) + aHigG Acr\* (10ng/ml)  
Washes: HCV Ag Assay Transfer: HIV Ag Devlot5, Final wash: HCV Ag final wash prep B1/2000  
SDB: HCV Ab (BA52C)  
S/A (1023) configuration: HCV

	SubA	SubB	Mean	P/N
PC (Ab)	3454	3656	3555	4.84
PC (Ag)	5303	6014	5658.5	7.71
PC (Ag)	4288	3722	4005	5.46
HC (99900)	637	831	734	
E2 1/20	12480	13082	12786	17.42
ProMed 9990196	11449			15.60
9990164	15	No conjugate was added		13.71
9990162	10080		10060	18.97
9990212	13525		13925	1.30
Sero-Tec panels #3	958		958	
4	2347		2347	4.53
5	3400		3400	4.53
6	4673		4673	4.81
7	4285		4285	4.15
8	3045		3045	

\*Materials: Code/List/Desc/Lot see RB: 68160

SIGNATURE W. J. Jiang DATE \_\_\_\_\_  
WITNESSED BY C. J. Johnson DATE \_\_\_\_\_

PROJECT \_\_\_\_\_

EXP. OR CODE NO. Cont. from page #17

1023      6      A      N/A      09/15/00      11:10:02      L JIANG  
BC SEROCONVERSION

ups	Conjugate	NC	SubA	SubB	Mean	P/N
11-28+NS3NS4+C11-14	50ng/ml + 7ng/ml Cl-70, v. High * 5751	452	452	456	454.00	17.13
		E2 (1/20)	7753	7804	7778.50	9.77
		PC (Ag)	4462	4407	4434.50	16.76
		9990212	7611		7611.	15.15
		9996196	6878		6878	11.31
		9996164	5133		5133	2.77
		Sero-Tec panel #3	1257		1257	2.77
		4	2540		2540	6.32
		5	2870		2870	10.83
		6	4917		4917	12.32
		BBI HCv sero 907 #1	5595		5595	5.96
		2	2707		2707	5.96
		3	2614		2614	5.76
		4	2701		2701	5.95
		5	2343		2343	5.16
		6	4443		4443	9.79
		7	8147		8147	17.94

ups	Conjugate	NC	SubA	SubB	Mean	2ng/ml P/N	7ng/ml P/N
11-28+NS3NS4+C11-14	50ng/ml + 2ng/ml	277	277	246	261.50	10.92	17.13
		E2 (1/20)	2831	2879	2855.00	15.89	9.77
		PC (Ag)	4213	4099	4156.00	10.60	16.76
		9990212	2773		2773	8.60	15.15
		9996196	2249		2249	7.33	11.31
		9996164	1918		1918	2.77	2.77
		Sero-Tec panel #3	2299		2299	8.79	6.32
		4	3002		3002	11.48	10.83
		5	5112		5112	19.55	12.32
		6	3754		3754	14.36	5.96
		BBI HCv sero 907 #1	3363		3363	12.86	5.76
		2	2230		2230	8.53	5.95
		3	2404		2404	9.19	5.16
		4	1743		1743	6.67	9.79
		5	2084		2084	7.97	17.94
		6	1566		1566	5.99	
		7					

*Lijiang*

SIGNATURE *Lijiang* DATE \_\_\_\_\_

WITNESSED BY *Arthur Brown* DATE \_\_\_\_\_

PROJECT \_\_\_\_\_  
 EXP. OR CODE NO. Cont. from page # 18

**BOSTON BIOMEDICA, INC.**  
 Anti-HCV Seroconversion Panel (PHV907)  
 HCV Genotype 1A

Panel Member	Blood Date	Day No.	ABIGIT S/CU	ORTHO HCV 3.0 S/CU	Combo data S/CU	Combo data ng/ml	Abbott At-Only S/CU	Abbott At-Only ng/ml	Reverse Amplico IU/ml
PHV907-1		0	0.1	0.0	12.3	14.4	25.68	25.68	3 x 10e6
PHV907-2		4	0.1	0.0	9.0	12.9	20.41	20.41	2 x 10e6
PHV907-3		7	0.1	0.0	9.8	9.9	17.98	17.98	1 x 10e6
PHV907-4		13	0.2	0.1	6.0	8.2	15.98	15.98	1 x 10e6
PHV907-5		18	0.8	0.5	5.2	9.7	6.88	6.88	1 x 10e6
PHV907-6		21	1.4	1.0	9.8	8.0	7.90	7.90	1 x 10e6
PHV907-7		184	>6.0	>6.0	18.0	6.0	0.70	0.70	nd

Data above demonstrates on seven member seroconversion panel, that HCV RNA and HCV Antigens can be detected from the first bleed date through the sixth bleed date, but the seventh bleed date is negative for HCV antigen. The antibody tests Ortho 3.0 and Abbott 3.0 failed to detect antibodies in the first five bleed dates ( ) through ( )...

The combo test detected exposure to HCV for all seven bleed dates.

SIGNATURE W. J. ... DATE \_\_\_\_\_  
 WITNESSED BY Cathy ... DATE \_\_\_\_\_



PROJECT PRISM HCV Ag/Ab combo  
EXP. OR CODE NO. HCV combo Assay Random Donor Population  
Reagents: Same as exp # 6816017.

1023  
OMBO SMALL POP  
A  
N/A  
08/18/00 11:00:17 L. JIANG  
HCV Combo Assay Population

SubA	SubB	Mean	PN	Cutoff (3nc)	SICO	Cutoff (2.5nc)	SICO	Cutoff (2.33nc)	SICO
3737	3884	3795.6	16.30	744	6.12	620	6.12	677.84	6.67
2949	2785	2867	11.66		4.92		4.92		4.98
214	282	248							0.31
162									0.28
163									0.26
282									0.61
4	172								0.30
5	265								0.28
6	172								0.46
7	271								0.30
8	329								0.47
9	200								0.67
10	247								0.32
11	196								0.43
12	248								0.34
13	432								0.43
14	214								0.70
15	161								0.37
16	218								0.28
17	140								0.28
18	218								0.37
19	209								0.24
20	209								0.29
21	200								0.36
22	186								0.23
23	248								0.37
24	209								0.49
25	291								0.48
26	241								0.34
									0.36
									0.32
									0.43
									0.40
									0.38
									0.47
									0.60
									0.42

SIGNATURE [Signature] DAT  
WITNESSED BY [Signature] DATE